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HHV-6 Reactivation and Associated Sequelae after Hematopoietic Cell Transplantation

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Human herpesvirus 6 (HHV-6) reactivation has been associated with acute graft-versus-host-disease (aGVHD), cytomegalovirus reactivation, and mortality after allogeneic hematopoietic cell transplantation (HCT), but previous studies have yielded inconsistent results. We performed a large prospective study of allogeneic HCT recipients in order to more definitively define the relationships between HHV-6 and these important outcomes. Plasma specimens were collected prospectively from 315 allogeneic HCT recipients and tested for HHV-6 DNA at baseline and twice weekly for 12 weeks. Cox proportional hazards models were used to evaluate the time-dependent associations between HHV-6 reactivation and the targeted outcomes. HHV-6 was detected in 111 of 315 patients (35%) at a median of 20 days after HCT. HHV-6 reactivation was associated with subsequent cytomegalovirus reactivation (adjusted hazard ratio [aHR], 1.9; 95% confidence interval [CI], 1.3-2.8; $P = .002$). High-level HHV-6 (>1,000 HHV-6 DNA copies/mL) was associated with subsequent grades II to IV aGVHD (aHR, 2.4; 95% CI, 1.60-3.6; $P < .001$). High-level HHV-6 reactivation was also associated with nonrelapse mortality (aHR, 2.7; 95% CI, 1.2-6.3; $P = .02$). HHV-6 reactivation was independently and quantitatively associated with increased risk of subsequent cytomegalovirus reactivation, aGVHD, and mortality after HCT. A randomized antiviral trial is warranted to establish causality between HHV-6 and these endpoints and to determine if reducing HHV-6 reactivation will improve outcome after HCT.

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INTRODUCTION

Human herpesvirus 6 (HHV-6) infects 90% to 100% of individuals during early childhood [1]. After primary infection, HHV-6 establishes a latent infection in hematopoietic reservoirs. This latent infection can become active in settings of severe immunosuppression, especially hematopoietic cell transplantation (HCT). Prior studies show that HHV-6 reactivates in approximately 40% of patients after HCT [2-4], and reactivation has variably been associated with important outcomes, including cytomegalovirus (CMV) reactivation [5], acute graft-versus-host-disease (aGVHD) [2,6,7], and increased mortality [4,6]. Whether HHV-6 is actually causally associated with these problems remains controversial.

We performed a large prospective study of HHV-6 in HCT recipients in an effort to better understand the relationships between HHV-6, CMV, aGVHD, and mortality in these patients.

MATERIALS AND METHODS

The data presented in this report were generated in part from a prospective study designed to evaluate the associations between HHV-6 reactivation and neuropsychiatric and neurocognitive outcomes [8]. The protocol was approved by the Fred Hutchinson Cancer Research Center Institutional Review Board.

Patients

Patients of all ages undergoing allogeneic HCT from January 2005 through August 2008 were eligible for enrollment. Those with limited English proficiency were excluded due to the frequent neuropsychiatric and neurocognitive assessments required for the

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parent study. The study was presented to 880 patients during the pretransplantation evaluation, and 474 were preliminarily enrolled (Figure 1). Of the 474 patients, a total of 152 withdrew or were deemed ineligible before contributing data, leaving 322 patients who contributed data. Six patients with HHV-6 DNA levels suggestive of chromosomal integration [9] (determined a priori as increasing HHV-6 plasma DNA levels during the first 2 weeks after HCT and persistent levels ≥100 copies per/mL in ≥80% of subsequent plasma samples) were excluded from analyses. An additional patient, who contributed only baseline data, was also excluded. Of the 315 included patients, nearly all (n = 308; 98%) were followed for ≥4 weeks after HCT or until death.

Clinical Care

Participation in this study had no impact on clinical decisions, including those involving conditioning regimens, type of transplantation, aGVHD prophylaxis and treatment, or administration of antimicrobials. There were no recipients of T cell-depleted stem cell grafts. CMV reactivation was monitored and treated per clinical standards of care. From the initiation of the study through February 2007, the primary mode of CMV screening was CMV antigenemia. After this point, plasma CMV PCR became the primary means of CMV screening. Patients were tested weekly for evidence of CMV reactivation through approximately day 100 after HCT. A pre-emptive antiviral therapy approach was followed [10,11]. Ganciclovir was the first-line antiviral postengraftment, and foscarnet was the second.

Study Procedures

Baseline (pre-HCT) and twice-weekly plasma specimens were collected through day 84 post-HCT for HHV-6 testing. A total of 6,255 specimens were obtained (85% of planned). Patients were followed through day 200 for mortality.

Clinical Data and Definitions

Demographic, clinical, and laboratory information was collected from clinical records and databases.

Underlying disease was categorized as "less advanced" or "more advanced" (Table 1) [12].

Medical comorbidity was defined and categorized using a validated scale [13].

Pretransplantation lymphopenia was assessed at the last lymphocyte count obtained before starting conditioning chemotherapy and was defined as a lymphocyte count <600 (approximate lowest quartile).

Conditioning regimens were categorized as "myeloablative," "nonmyeloablative," or "reduced intensity." A variety of cytoreductive regimens were used; the most common regimens are reported in Table 1 by myeloablative category.

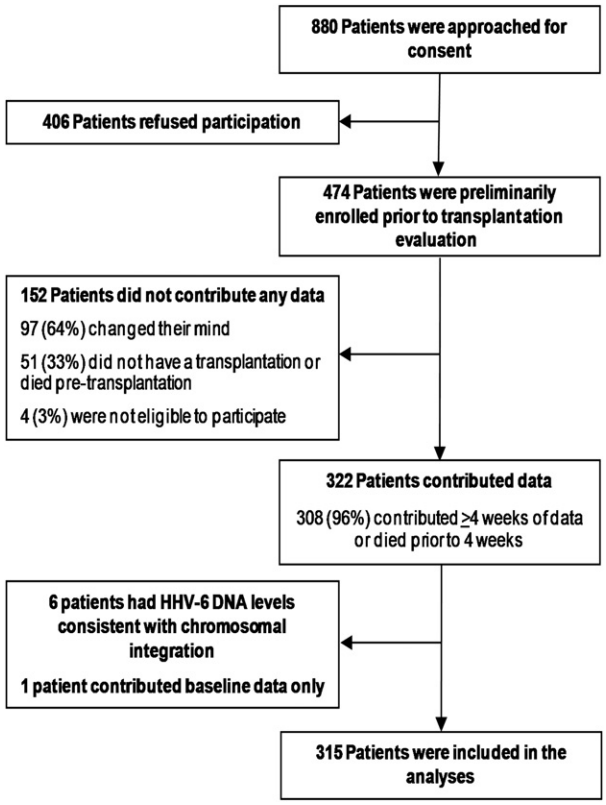

Figure 1. Consort diagram.

HHV-6 reactivation was defined as any level of plasma HHV-6 DNA.

High-level HHV-6 reactivation was defined as ≥1,000 HHV-6 DNA copies/mL plasma. This level was chosen because it is a threshold for CMV that is commonly used to initiate use of pre-emptive antiviral therapy. In addition, in the context of this study, it is close to the median maximum level (873 HHV-6 copies/mL plasma).

CMV reactivation was defined as any level of plasma CMV DNA or whole blood antigenemia.

High-level CMV reactivation was defined as ≥1,000 CMV DNA copies/mL plasma or 10 cells/high-powered field of CMV antigenemia.

Acute graft-versus-host disease (aGVHD) grades and organ-specific types (skin, gastrointestinal, and liver) and stages were categorized as previously described by a single investigator (P.J.M.) blinded to HHV-6 study results [14].

Chronic graft-versus-host disease (cGVHD) was categorized as previously described [15].

Overall mortality was defined as mortality occurring for any reason.

Nonrelapse mortality was defined as mortality occurring for reasons other than relapse in patients receiving myeloablative HCT or for reasons other than relapse or progression of underlying disease in patients receiving nonmyeloablative HCT.

Table 1. Demographic and Clinical Characteristics of the Cohort, Overall and Stratified by ever having HHV-6 Reactivation

	Overall (n = 315) n (%)	HHV-6 Reactivation (n = 315)	
		No (n = 204)	Yes (n = 111)
Age			
≤20 yrs	63 (20)	33 (16)	30 (27)
21-30 yrs	22 (7)	12 (6)	10 (9)
31-40 yrs	31 (10)	22 (11)	9 (8)
41-50 yrs	51 (16)	34 (17)	17 (15)
>50 yrs	148 (47)	103 (50)	45 (41)
Female sex	126 (40)	82 (40)	44 (40)
Race/ethnicity			
White	257 (82)	172 (84)	85 (76)
Hispanic	10 (3)	6 (3)	4 (4)
Native American	6 (2)	3 (2)	3 (3)
Other	42 (13)	23 (11)	19 (17)
CMV donor/recipient serostatus			
Donor+/recipient+	80 (25)	48 (24)	32 (29)
Donor+/recipient−	31 (10)	21 (10)	10 (9)
Donor−/recipient+	92 (29)	57 (28)	35 (31)
Donor−/recipient−	112 (36)	78 (38)	34 (31)
Positive HSV serostatus	265 (85)	167 (83)	98 (88)
Medical comorbidity			
Low	93 (30)	52 (26)	41 (37)
Moderate	95 (30)	68 (33)	27 (24)
High	127 (40)	84 (41)	43 (39)
More advanced underlying disease*	174 (55)	109 (53)	65 (59)
TBI			
≥1,200 cGy	72 (23)	36 (17)	36 (33)
≤400 cGy	127 (40)	81 (40)	46 (41)
None	116 (37)	87 (43)	29 (26)
Conditioning regimen			
Myeloablative†	169 (54)	110 (54)	59 (53)
Nonmyeloablative‡	112 (35)	79 (39)	33 (30)
Reduced intensity§	34 (11)	15 (7)	19 (17)
HLA match			
Matched related	98 (31)	74 (36)	24 (22)
Matched unrelated	142 (45)	95 (47)	47 (42)
Mismatched related‖	10 (3)	4 (2)	6 (5)
Mismatched unrelated¶	65 (21)	31 (15)	34 (31)
Stem cell source			
Bone marrow	61 (19)	34 (17)	27 (24)
Cord blood	21 (7)	6 (3)	15 (14)
Peripheral blood stem cell	233 (74)	164 (80)	69 (62)

HHV indicates human herpesvirus; CMV, cytomegalovirus; HSV, herpes simplex virus; TBI, total body irradiation.

*"More advanced" underlying disease refers to diagnoses other than acute myeloid leukemia, acute lymphoblastic leukemia, or lymphoma in first remission, chronic myeloid leukemia in chronic phase, and refractory anemia without excess blasts.

†Myeloablative regimens included: any regimen containing ≥800 cGY TBI, any regimen containing carmustine/etoposide/cytarabine/melphalan (BEAM), or any regimen containing busulfan/cyclophosphamide with or without antithymocyte globulin. The most common regimens included busulfan and cyclophosphamide (n = 73), cyclophosphamide and >1,200 cGY TBI (n = 47), and cyclophosphamide, fludarabine, and 1,320 cGY TBI (n = 13).

‡The nonmyeloablative regimen was fludarabine 90 mg/m2 +/− TBI <300 cGY. The most common regimens included fludarabine and 200 cGY TBI (n = 76) and fludarabine and 300 cGy TBI (n = 9).

§All other regimens were considered reduced intensity. The most common regimen was treosulfan and fludarabine (n = 17).

‖Allele or antigen mismatch: 9/10 (n = 4), 6/10 (n = 1), and 5/10 (n = 5).

¶Allele or antigen mismatch: 9/10 (n = 40), 8/10 (n = 4), cord blood transplantation (CBT) recipients (n = 21) - all CBTs were mismatched, and a subset (n = 16) was double CBTs.

Antivirals that might affect CMV or HHV-6 activity were categorized as "low activity" (acyclovir) or "high activity" (foscarnet, ganciclovir, or cidofovir).

Laboratory Procedures

Individuals blinded to patients' clinical status performed PCR analyses as previously described [8]. Detection of 1 copy of HHV-6 DNA/reaction (25 copies/mL of plasma) was considered a positive specimen. All HHV-6 DNA identified by PCR was typed as HHV-6 A or B [16].

Statistical Analyses

Cox proportional hazards models were used to evaluate the impact of HHV-6 on the hazards of subsequent occurrence of each of the endpoints of interest for this study: CMV reactivation, aGVHD, cGVHD, and mortality. Mortality was assessed through day 200, whereas CMV and aGVHD were assessed through 100 days, and cGVHD endpoints were assessed both through day 100 and 1 year. Both grades II to IV and III to IV overall aGVHD were evaluated. In addition, organ-specific aGVHD was examined using the stages that specifically inform overall aGVHD grades II to IV and III to IV: stages 3 to 4 and 4 skin subtypes and stages 1 to 4 and 2 to 4 liver and gastrointestinal subtypes. The number of observations was insufficient to perform multivariable analyses of grade IV overall aGVHD. We also evaluated a composite endpoint of any mortality, grade II to IV aGVHD, or any level of CMV reactivation to evaluate the impact HHV-6 reactivation has on the likelihood of remaining alive and free of aGVHD and CMV reactivation through 200 days after HCT. The key risk factor of interest for all analyses was HHV-6 reactivation, modeled as a time-dependent covariate and coded as positive using 2 definitions (modeled separately): (1) any level of detectable HHV-6 and (2) HHV-6 ≥1,000 copies/mL plasma. Additional covariates included baseline demographic and clinical variables (Table 1). When HLA match was analyzed as a covariate, 3 strata were used: "matched (10 of 10 allele and antigen matched) related" versus "matched unrelated" versus "mismatched related" plus "mismatched unrelated." Mismatched related and mismatched unrelated were combined into one category due to the small number of mismatched related cases (n = 10). We also evaluated pretransplantation lymphopenia as defined above in each of the models. In addition, the dose of CD34-postive cells, use of antithymocyte globulin, female donor/male recipient status, and prior HCT were investigated in models for aGVHD. Administration of antiviral medications was also examined as a covariate for CMV reactivation. As an initial variable selection step, each factor was evaluated in a bivariable model with HHV-6 and

Table 2. Multivariable Models Evaluating HHV-6 as a Predictor of CMV Reactivation by Day 100 after HCT in Patients Seropositive for CMV (Donor or Recipient)

	Any Level CMV		High-level CMV	
	aHR (95% CI)	*P* Value	aHR (95% CI)	*P* Value
Models with any level HHV-6				
HHV-6 reactivation	1.88 (1.26-2.82) Covariates*,†,‡,§	.002	1.14 (0.61-2.13) Covariates†,‡,‖	.69
Models with high-level HHV-6				
HHV-6 reactivation	1.56 (0.96-2.54) Covariates*,†,‡,§	.08	3.11 (1.52-6.36) Covariates†,‡,‖,¶	.002

HHV-6 indicates human herpesvirus-6; CMV, cytomegalovirus; HCT, hematopoietic cell transplantation; aHR, adjusted hazard ratio; CI, confidence interval.
Covariates included in the final multivariable models: *recipient CMV positive, †stem cell source, ‡myeloablative transplant, §pretransplantation lymphocyte count, ‖HLA match, and ¶age.

considered eligible for the multivariable model if the 2-sided *P* value was < .20 or if its inclusion modified the effect of HHV-6 by >10%. Once included in a full multivariable model, in stepwise evaluation, each factor was retained if its *P* value was < .10 or if its inclusion modified the effect of HHV-6 by >10%. Potential interactions between HHV-6 and other key variables were formally assessed when indicated. Interaction terms between HHV-6 and aGVHD were evaluated in mortality models. We were unable to explore interaction between HHV-6 and stem cell source because most patients who underwent cord blood transplantation (CBT) had HHV-6 reactivation. Instead, analyses were repeated on a cohort without the CBT recipients. All reported *P* values are 2-sided and considered significant if $P < .05$.

RESULTS

Of the 315 patients included in analyses, 63 (20%) were <21 years of age, 169 (54%) received myeloablative conditioning, and 217 (69%) received cells from unrelated or HLA-mismatched related donors (Table 1). The stem cell source was growth factor-mobilized peripheral blood cells for 233 (74%), bone marrow for 61 (19%), and cord blood for 21 (7%). Of the 21 CBT recipients, 76% received double CBTs.

HHV-6

HHV-6 was detected in 111 (35%) of the 315 patients by day-84 post-HCT. The median time to first detection among those with reactivation was 20 days (interquartile range [IQR], 15-28 days) after HCT. The median duration of the first episode of HHV-6 detection, from the first positive through the last consecutive positive, was 4 days (IQR, 1-11 days). The median maximum DNA level was 873 copies/mL (IQR, 175-4,580), and the HHV-6 DNA level was ≥1,000 copies/mL in 53 (17%). HHV-6 was detected in 16 (76%) of the 21 CBT recipients, and in all cases, the HHV-6 DNA level was ≥1,000 copies/mL. All detected HHV-6 was type B.

HHV-6 and CMV

Only the patients with donor or recipient CMV seropositive status (n = 203) were included in analyses of CMV reactivation. The distribution of HLA match categories and stem cell source among these 203 patients was similar to the overall cohort (data not shown). Any level of CMV reactivation occurred in 128 of 203 patients (63%) who were donor or recipient CMV-seropositive. High-level CMV reactivation (antigenemia ≥10 cells per high-powered field or CMV DNA level ≥1,000 copies /mL) occurred in 54 (26%), and CMV disease occurred in 7 (3%). First detection of any level of CMV reactivation occurred at a median of 36 days (IQR, 25-53 days) after HCT. In patients with donor or recipient CMV seropositive, HHV-6 reactivation (any DNA level) was independently associated with increased risk of subsequent CMV reactivation of any level (adjusted hazard ratio [aHR], 1.88; 95% confidence interval [CI], 1.26-2.82; $P = .002$) but was not associated with high-level CMV reactivation (Table 2). In contrast, high-level HHV-6 reactivation (>1,000 copies DNA/mL) was not significantly associated with CMV reactivation at any level (Table 2), but it was strongly associated with increased risk of subsequent high-level CMV reactivation (aHR, 3.11; 95% CI, 1.52-6.36; $P = .002$). The addition of grades II to IV or grades III to IV aGVHD to the multivariable models of HHV-6 and CMV reactivation did not markedly change the HR point estimates or statistical significance for HHV-6, even though the aGVHD covariates were strong predictors of CMV reactivation (data not shown). Analyses were repeated excluding the CBT recipients, and the results were not meaningfully different (data not shown). The number of observations was insufficient to evaluate HHV-6 reactivation as a predictor of CMV disease. In addition, there were few patients who received ganciclovir, foscarnet, or cidofovir early after HCT; therefore, we were unable to evaluate the impact of these antivirals on risk of CMV or HHV-6 reactivation or HHV-6 viral DNA levels.

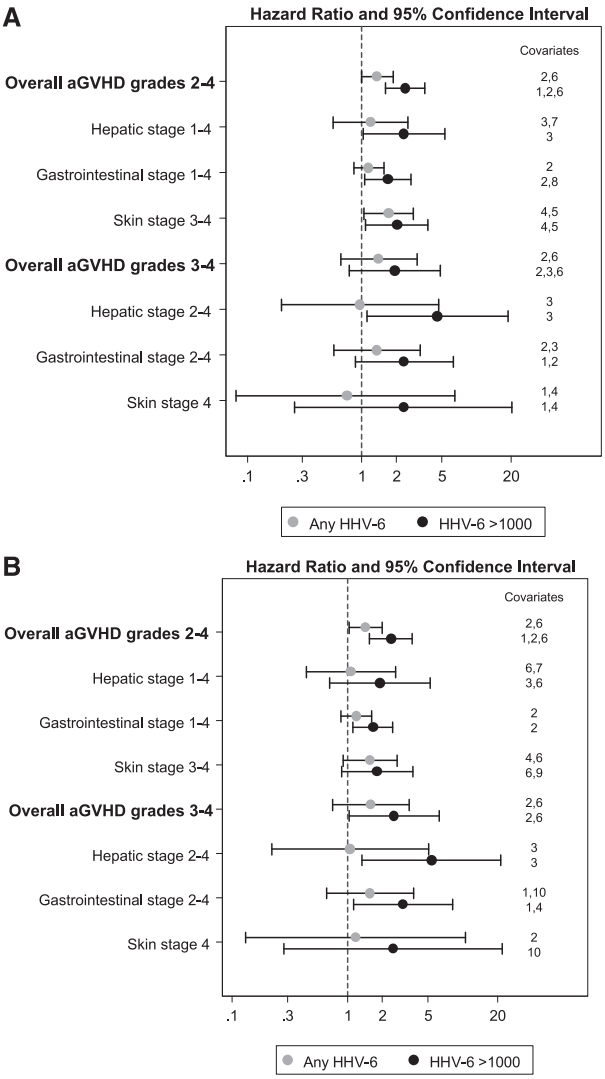

Figure 2. Results from multivariable models evaluating human herpesvirus 6 (HHV-6) reactivation as a risk factor for subsequent acute graft-versus-host disease (aGVHD) by day 100. (A) Full cohort. (B) Excluding cord blood recipients. Covariates included in the final multivariable models: [1]age, [2]conditioning regimen, [3]stem cell source, [4]sex, [5]underlying disease, [6]HLA match, [7]cytomegalovirus (CMV) serostatus, [8]CD34 dose, [9]comorbidity index, and [10]female donor/male recipient.

HHV-6 and aGVHD

Grade II to IV aGVHD was diagnosed in 240 patients (76%) with onset of symptoms or signs at a median of 27 days (IQR, 19-41 days) after transplantation. Grade III to IV aGVHD was diagnosed in 50 patients (16%) with onset of symptoms at a median of 22 days (IQR, 12-28 days) after transplantation. Among the patients who developed HHV-6 and subsequent aGVHD, the interval between the first detection of HHV-6 and onset of the first symptoms or signs of grade II to IV aGVHD was a median of 11 days (IQR, 5-25 days; n = 63) and between HHV-6 and grade III to IV aGVHD was a median of 4.5 days (IQR, 3-7 days; n = 12). Similar intervals were observed between high-level HHV-6 and the aGVHD outcomes (data not shown). In a multivariable model, we observed a nonsignificant but suggestive association between HHV-6 reactivation and subsequent grade II to IV overall aGVHD (aHR, 1.36; 95% CI, 0.99-1.88; $P = .06$). The association was strengthened when high-level HHV-6 reactivation was evaluated (aHR, 2.39; 95% CI, 1.60-3.56; $P < .001$). HHV-6 reactivation was not, however, significantly associated with grade III to IV overall aGVHD (Figure 2).

HHV-6 reactivation was evaluated in multivariable models as a risk factor for organ-specific subtypes of aGVHD: skin, gastrointestinal, and liver aGVHD. A significant association was demonstrated between HHV-6 and stages 3 to 4 skin aGVHD (aHR, 1.71; 95% CI, 1.04-2.81; $P = .04$), and the association was strengthened when high-level HHV-6 reactivation was evaluated (aHR, 2.01; 95% CI, 1.08-3.75; $P = .03$; Figure 2). High-level HHV-6 was associated with increased risk of both subsequent stages 1 to 4 and 2 to 4 hepatic aGVHD (aHR, 2.34; 95% CI, 1.03-5.30; $P = .04$ and aHR, 4.53; 95% CI, 1.10-18.68; $P = .04$, respectively; Figure 2). High-level HHV-6 was also associated with increased risk of subsequent stages 1 to 4 gastrointestinal aGVHD (aHR, 1.68; 95% CI, 1.05-2.68; $P = .03$; Figure 2).

Analyses of aGVHD excluding CBT recipients produced similar results (Figure 2), although associations became statistically significant for any level of HHV-6 and grades II to IV overall aGVHD as well as for high-level HHV-6 and grades III to IV overall aGVHD. In addition, statistical significance was diminished for associations of high-level HHV-6 and stages 1 to 4 hepatic aGVHD as well as for both any level and high-level HHV-6 and stages 3 to 4 skin aGVHD.

HHV-6 and cGVHD

HHV-6 reactivation was not associated with cGVHD by day 100 in either univariate (HR, 1.19; 95% CI, 0.84-1.69; $P = .33$) or multivariate (HR, 1.18; 95% CI, 0.82-1.71; $P = .38$) analyses. Similar results were obtained at the 1-year time point (data not shown).

HHV-6 and Mortality

Overall, 60 of the 315 patients (19%) died by day 200 after HCT and in 40 patients (13%), the cause of death was considered to be nonrelapse mortality. Of the 40 patients with nonrelapse mortality, 17 (43%) had a fatal infection with (n = 7) or without (n = 10) aGVHD. Other causes included multiorgan failure (10; 25%), respiratory failure (3; 8%), cerebrovascular event (3; 8%), and other causes (7; 18%).

High-level HHV-6 reactivation was independently associated with increased risk of nonrelapse mortality (aHR, 2.70; 95% CI, 1.15-6.34; $P = .02$). There was a suggestion that both any level of HHV-6 reactivation and high-level HHV-6 reactivation were associated with an increased risk of overall

Table 3. Multivariable Models Evaluating HHV-6 (Modeled as any Level and Level >1,000 Copies/mL) as a Risk Factor of the Outcome Mortality (Evaluated as Overall Mortality and as Nonrelapse Mortality)

	Overall Mortality by Day 200		Non-relapse Mortality by Day 200	
	aHR (95% CI)	*P* Value	aHR (95% CI)	*P* Value
Models with any level of HHV-6				
Any HHV-6 alone				
Any HHV-6	1.69 (0.99-2.87) Covariates*,†,‡	.053	1.74 (0.88-3.41) Covariates*,†,‡,§,||,¶,**	.11
Any HHV-6 with aGVHD grades II-IV				
Any HHV-6	1.60 (0.93-2.75)	.09	1.73 (0.87-3.42)	.12
aGVHD grades II-IV	1.36 (0.71-2.58) Covariates*,†,‡	.35	1.61 (0.72-3.62) Covariates*,‡,¶	.25
Any HHV-6 with aGVHD grades III-IV				
Any HHV-6	1.42 (0.82-2.45)	.21	1.37 (0.70-2.70)	.36
aGVHD grades III-IV	2.88 (1.61-5.14) Covariates*,†,‡	<.001	4.39 (2.23-8.63) Covariates*,‡	<.001
Models with high-level HHV-6				
HHV-6 alone				
HHV-6 >1,000 c/mL	1.95 (0.99-3.86) Covariates*,†,‡,§,||,¶	.054	2.70 (1.15-6.34) Covariates*,‡,§,||,¶,††	.02
HHV-6 with aGVHD grades II-IV				
HHV-6 >1,000 c/mL	1.81 (0.90-3.62)	.10	2.50 (1.04-6.03)	.04
aGVHD grades II-IV	1.33 (0.69-2.57) Covariates*,†,‡,§,¶	.39	1.35 (0.59-3.09) Covariates*,‡,§,||,¶,††	.48
HHV-6 with aGVHD grades III-IV‡‡				
HHV-6 and aGVHD grades III-IV composite variable				
Neither HHV-6 or aGVHD grades III-IV	Reference		Reference	
HHV-6 >1,000 c/mL only	2.61 (1.22-5.62)	.014	3.61 (1.45-8.99)	.006
aGVHD grades III-IV only	4.42 (2.34-8.34)	<.001	7.02 (3.35-14.7)	<.001
HHV-6 >1,000 c/mL and aGVHD grades III-IV	2.50 (0.72-8.71) Covariates*,†,‡,§,¶	.15	4.36 (0.93-20.4) Covariates*,‡,¶,††	.06

HHV-6 indicates human herpesvirus-6; aHR, adjusted hazard ratio; CI, confidence interval; aGVHD, acute graft-versus-host disease.
Models were evaluated with and without aGVHD as a covariate (modeled as grade II-IV or grade III-IV) and with and without CMV reactivation as a covariate. CMV reactivation was not a significant predictor of mortality, and its inclusion in the model did not affect the HHV-6 point estimate or significance (data not shown).
Other covariates included in the final models: *age, †underlying disease, ‡ cytomegalovirus (CMV) serostatus, §myeloablative conditioning, ||comorbidity index, ¶stem cell source, **chronic GVHD, and ††race.
‡‡Due to the significant interaction between high-level HHV-6 reactivation and aGVHD grades III-IV for each mode ($P = .04$ for overall survival and $P = .05$ for nonrelapse mortality), a 4-level composite variable was evaluated in the final models including these factors to illustrate the varying association depending on whether each factor is present singly or in combination. The reference category for this variable is defined by having neither HHV-6 nor aGVHD grades III-IV. No effect modification was observed between any level HHV-6 and aGVHD grades II-IV, any level HHV-6 and aGVHD grades III-IV, or between high-level HHV-6 and aGVHD grades II-IV. Therefore, a composite variable was not required for these models.

mortality, although these findings were not statistically significant (Table 3). Significant interactions were observed between high-level HHV-6 and grades III to IV overall aGVHD for both overall survival ($P = .04$) and nonrelapse mortality ($P = .05$). Thus, we created a composite variable for high-level HHV-6 and grades III to IV aGVHD for use in the mortality models to illustrate the varying degrees of association depending on whether each factor was present singly or in combination (Table 3). When they occurred alone, both high-level HHV-6 and grades III to IV aGVHD were associated with increased risks of mortality compared to absence of the 2 conditions. Co-occurrence of high-level HHV-6 and grades III to IV aGVHD was also associated with increased risk of mortality compared with patients with neither condition; however, the level of risk was not significantly different from the risk associated with each condition alone. That is, among patients with grades III to IV aGVHD, addition of high-level HHV-6 did not result in a significantly increased risk of mortality, although the number of patients who experienced both high-level HHV-6 and grades III to IV aGVHD was relatively small (n = 13). Inclusion of grade II to IV aGVHD in the models did not have a large effect on the associations between HHV-6 and mortality. CMV reactivation was not associated with risk of subsequent mortality and did not significantly alter the associations between HHV-6 reactivation and mortality (data not shown). Analyses of mortality were also performed excluding the CBT recipients. Point estimates for the HRs were similar, but significance was diminished or lost across most categories (data not shown). Only high-level HHV-6 remained significantly associated with nonrelapse mortality (HHV-6 only: HR, 3.13; 95% CI, 1.22-8.03; $P = .02$).

HHV-6 and the Composite Endpoint

HHV-6 reactivation of any level was associated with the composite endpoint of any mortality, grades II to IV aGVHD, or any level of CMV reactivation (aHR, 1.47; 95% CI, 1.08-2.01; $P = .015$). Other

variables retained in the model included gender, pre-HCT CMV seropositivity in the donor or recipient, type of conditioning regimen, stem cell source, pre-transplantation lymphopenia, and HLA match between the donor and recipient. High-level HHV-6 appeared even more strongly associated with this composite outcome (aHR, 2.02; 95% CI, 1.35-3.01; $P < .001$). Patient age, stem cell source, type of conditioning regimen, HLA match between the donor and recipient, and pre-HCT positive serology for HSV remained in the final model.

DISCUSSION

In this prospective study of 315 HCT recipients, we found that HHV-6 reactivation was associated with increased risk of subsequent CMV reactivation, aGVHD, and mortality. Associations between HHV-6 and grades II to IV aGVHD, nonrelapse mortality, and high-level CMV reactivation were strengthened when high-level HHV-6 was evaluated.

Previous studies have shown an association between HHV-6 and aGVHD [2,17], of which several have used multivariable analyses in an attempt to control for potential confounders [4,6,7]. In the current study, we also evaluated organ-specific subtypes of aGVHD as outcomes. Results from these analyses suggest that HHV-6 may play a role in development of each of the aGVHD subtypes. It is interesting that the strength of the association between high-level HHV-6 and skin aGVHD was lessened when CBT recipients were removed from the analysis. Most of the 21 CBT recipients (76%) in our study received double CBTs, and these transplantations have been associated with greater risk of skin aGVHD [18]. This finding, along with the fact that 76% of the CBT recipients in our study had high-level HHV-6 reactivation, would explain these results. The association of HHV-6 reactivation with skin aGVHD raises the question of whether HHV-6 might actually trigger aGVHD or simply cause symptoms and signs that are then attributed to aGVHD. Others have demonstrated an association between HHV-6 and rash during the first month after HCT [19-22], and HHV-6 is known to cause a rash during primary acquisition [23]. Confirming the role of HHV-6 in aGVHD and distinguishing rash related to HHV-6 from rash related to aGVHD is necessary in order to target therapy appropriately.

Although a causal association between HHV-6 and aGVHD has not been established, certain data support the biologic plausibility of such a relationship. Specifically, in vitro and limited clinical data suggest that HHV-6 infection or reactivation may cause a proinflammatory or type I immune response, which may play an important role in the development of aGVHD. For example, a type I immune response polarization has been observed during HHV-6 infection of T cells in vitro [24], and small studies of HCT recipients have documented a proinflammatory cytokine response (primarily elevated IL-6 concentrations) associated with HHV-6 reactivation [25,26]. In parallel, a number of proinflammatory or type I cytokines (IL-2, sIL-2R, IL-5, IL-6, IL-10, IFN-gamma, IL-12, and IL-18) have been associated with aGVHD [27-30]. Although few studies exploring potential associations between HHV-6, the immune response, and aGVHD have been described, data from a small number of patients with HHV-6 reactivation after HCT have implied a temporal association between elevated cytokines and rash or aGVHD [26]. Further in vivo study of the effects of HHV-6 on the immune system may help elucidate the pathogenesis of HHV-6–related disease.

Seemingly in contrast to the concept of an HHV-6-induced proinflammatory response, other in vitro evidence suggests that HHV-6 reactivation might suppress the antiviral immune response, potentially through suppression of IL-12 production [31-33]. Such suppression of antiviral immune responses could potentially promote the HHV-6 infection itself, as well as other viral infections, including CMV. In solid-organ transplantation recipients, HHV-6 reactivation has been independently associated with CMV reactivation and disease [34,35]; whereas studies conducted in HCT populations have yielded variable results. In a study of 21 allogeneic HCT recipients, HHV-6 reactivation was associated with an absence of CMV-specific lymphocyte proliferative responses, and persistence of HHV-6 detection was associated with need for repeated courses of pre-emptive antiviral therapy against CMV during the first 6 months after HCT [36]. In contrast, a study of 68 allogeneic HCT recipients found that HHV-6 reactivation was associated with CMV reactivation in univariate analysis but not multivariable analyses [5]. In addition, detection of HHV-6 DNA in plasma did not seem to affect CMV-specific T cell immunity reconstitution as measured by intracellular cytokine staining. Therefore, the authors concluded that the severe immunosuppression that attends HCT leads to HHV-6 and CMV reactivation but that HHV-6 does not predispose to CMV reactivation or influence the course of active CMV infection [5]. There have been few published studies that have investigated the association between HHV-6 reactivation and CMV reactivation/disease in HCT recipients. The inconsistent results may be due in part to the small size of previous studies. Given the HR that we observed for the association between HHV-6 and CMV reactivation, relatively large studies involving several hundred patients, such as in our study, are needed to address this question. Despite having a large study, we did not have an adequate number of patients with HHV-6 and subsequent receipt of ganciclovir or foscarnet to assess the impact of these antivirals on HHV-6 levels and/or on CMV reactivation. A trial

testing the effect of an HHV-6-active antiviral on HHV-6 and CMV reactivation would likely clarify these issues.

In the present study, we also found that HHV-6 reactivation was independently associated with nonrelapse mortality, and borderline associations were noted between any-level and high-level HHV-6 reactivation and all-cause mortality. The association between HHV-6 reactivation and mortality in HCT has been reported previously in only a few studies [4,6]. Our data suggest that the association between HHV-6 and mortality may be mediated partly through aGVHD. Many complex pathways could link HHV-6 reactivation with increased risk of CMV reactivation, aGVHD, and mortality. It is also possible that other clinical conditions, such as central nervous system disease, pneumonitis, or bone marrow suppression might mediate the relationship between HHV-6 and mortality. The experimental design offered by a randomized controlled trial would likely shed light on these relationships. Our results also suggest that CBT recipients were important in driving the statistical significance of the associations we observed between HHV-6 and mortality. Thus, CBT recipients may be an important group to target for future intervention studies.

This study had many strengths, including the size of the cohort, the frequency and regularity of HHV-6 testing, and the systematic collection of outcome data. This allowed us to address important questions regarding the possible association of HHV-6 reactivation with CMV reactivation, GVHD, or mortality more definitively than previous smaller studies. However, due to the observational study design, we cannot conclude that HHV-6 reactivation is causally related to these clinical outcomes. The ubiquitous and persistent nature of HHV-6 infection poses significant challenges in establishing causal associations between viral reactivation and disease, especially in immunocompromised populations with multiple complex medical problems. An antiviral intervention trial would provide the experimental data required to determine causality.

In summary, we demonstrated an independent and quantitative association between HHV-6 reactivation and the outcomes of CMV reactivation, aGVHD, and mortality. A randomized antiviral trial is warranted to determine if reducing HHV-6 reactivation will reduce the incidence of these outcomes after HCT.

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